



EXPRESS MAIL NO. EV064842193US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Orest W. Blaschuk et al.
Application No. : 09/185,908
Filed : November 3, 1998
For : COMPOUNDS AND METHODS FOR MODULATING
CLAUDIN-MEDIATED FUNCTIONS

Examiner : Amy DeCloux, Ph.D.
Art Unit : 1644
Docket No. : 100086.409

Commissioner for Patents
Washington, DC 20231

DECLARATION OF JAMES MATTHEW SYMONDS UNDER 37 CFR § 1.132

The undersigned, James Matthew Symonds, hereby declares:

1. I am a Project Manager at Adherex Technologies, Inc., the assignee of the subject application.

2. I have reviewed the Office Action dated February 11, 2002 in the subject application, including the rejection under 35 U.S.C. § 112, first paragraph, and provide this Declaration for the purpose of assisting the Examiner in evaluating the teachings of the specification and to confirm that the presently claimed agents can modulate cell adhesion.

3. The experiments presented herein were performed by me, by those under my direct supervision, or are within my personal knowledge.

4. EFFECT OF REPRESENTATIVE MODULATING AGENTS ON ELECTRICAL RESISTANCE ACROSS CELL MONOLAYER


Madin Darby canine kidney (MDCK) cells were plated in Millicells (Millipore, Bedford, MA), at a density of 300,000 cells per Millicell, and cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma, St. Louis, MO) containing 5% fetal calf serum (Sigma, St. Louis, MO) until monolayers formed. Monolayers were exposed to the modulating agent dissolved in medium. The electrical resistance was measured using the EVOM device (World Precision Instruments, Sarasota, FL). At the time of measurement, fresh medium, with or without the modulating agent, may be added to the Millicells.

Appendix 1 shows the mean electrical resistance across MDCK cell monolayers cultured for 18 hours in medium alone (Control), medium containing N-Ac-WKIYSYAGDN-NH₂ (Peptide 118) or H-WKIYSYAGDN-NH₂ (Peptide 119) at a concentration of 0.5 mg/ml. Duplicate measurements were taken, and error bars represent the standard deviation. Peptide 118 reduced the electrical resistance across the monolayer, while peptide 119 did not change the electrical resistance across the monolayer relative to the control.

Appendix 2 shows the mean electrical resistance across MDCK cell monolayers cultured for 24 hours in medium alone (Control) or medium containing N-Ac-WKIYSYAGDN-NH₂ (Peptide 118) at various concentrations. Peptide 118 reduced the electrical resistance across the monolayer in a dose dependent manner.

These results demonstrate the ability of modulating agents to inhibit the formation of tight junctions in epithelial cells (i.e., modulate cell adhesion), as well as the effect of the N-Ac group of activity of this particular modulating agent.

5. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.



James Matthew Symonds

MAY 10, 2002
Date

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